7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring 1,4-dioxane, its metabolites, and other biomarkers of exposure and effect to 1,4-dioxane. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

Levels of 1,4-dioxane in environmental and biological samples are determined analytically by gas chromatography-mass spectrometry (GC-MS) or gas chromatography-flame ionization detection (GC-FID). The determination of 1,4-dioxane at parts-per-billion (ppb or µg/L) concentrations in samples where water is present (e.g., water, soil, sediment, and tissues) is difficult. This is because of the high solubility of 1,4-dioxane in water. As a polar volatile organic compound (VOC), 1,4-dioxane has a low purge efficiency from water compared to non-polar VOCs. Consequently, 1,4-dioxane has a poor purge-and-trap GC-MS response. The purge-and-trap technique also suffers from interferences by some substances. 1,4-Dioxane gives poor response with headspace sample introduction due to its low volatility from water. The partition coefficients for 1,4-dioxane lead to low recoveries in single contact liquid-liquid extraction (LLE), and very large solvent-to-water ratios are needed to achieve acceptable recoveries (Draper et al. 2000). Because of these limitations, alternative techniques have been developed to improve the determination of 1,4-dioxane. Methods have been developed to extract 1,4-dioxane extracted from the aqueous phase using solid phase extraction (SPE) followed by desorption with an organic solvent, heated purge-and-trap with salting out, azeotropic distillation; and continuous LLE. Isotopic dilution has also been used to correct for variability in MS instrument response.

7.1 BIOLOGICAL MATERIALS

Methods for the specific analysis of 1,4-dioxane and its metabolites in biological tissues and fluids are limited. Since the human body rapidly metabolizes 1,4-dioxane to 1,4-dioxane-2-one and HEAA, the metabolites of 1,4-dioxane may be used as biomarkers of exposure to 1,4-dioxane (Young et al. 1976).

Using heated headspace technique, 1,4-dioxane was determined in blood or urine (e.g., 4–5 mg) by heating a sample in a sealed tube to 120 °C. Volatiles from this sample were then analyzed by gas chromatography with the limit of detection being 0.01–0.02 µg (Royal Society of Chemistry 1988).

Groves et al. (1997) described the analysis of organic vapors in exhaled breath, which could provide information about occupational exposures to 1,4-dioxane. Analysis was conducted using an array of four polymer-coated surface-acoustic-wave (SAW) sensors and an adsorbant preconcentrator for rapid breath analysis. The adsorbant used in the preconcentrator was a porous styrene-divinylbenzene resin. Limits of detection range from 3.7 to 10.2 µg/L for 1,4-dioxane.

Biomarkers of exposure to 1,4-dioxane are the urinary metabolites, 1,4-dioxane-2-one and HEAA (Royal Society of Chemistry 1988). Young et al. (1976) described a method for detection of 1,4-dioxane and HEAA in urine. Urine samples were treated with hydrochloric acid/methanol to convert HEAA to its methyl ester. Samples were then directly injected into a GC-MS for simultaneous analysis of 1,4-dioxane and HEAA. The detection limits were 0.07 and 0.1 µg/mL, respectively.

Analytical methods for the determination of 1,4-dioxane and its metabolites in biological samples are given in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

NIOSH 1602 is used to determine the concentration of 1,4-dioxane in a 10-L air sample by GC-FID. Samples are collected by drawing air through a solid sorbent tube containing coconut shell charcoal. The flow rate is between 0.01 and 0.2 L/minute for a total sample size of 0.5–15 L. 1,4-Dioxane is eluted from the solid sorbent with agitation using carbon disulfide. The carbon disulfide eluent sample is then injected directly into the GC-FID. Detection limits are 0.01 mg per sample (NIOSH 1994).

Table 7-1. Analytical Methods for Determining 1,4-Dioxane in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood or urine	Heat sample in a sealed tube to 120 °C; inject headspace into GC	GC	0.01–0.02 µg	No data	Royal Society of Chemistry 1988
Exhaled breath	Pre-concentrate on breath sample on porous styrene-divinylbenzene resin	Four polymer-coated surface-acoustic-wave (SAW) sensors	3.7–10.2 µg/L	No data	Groves et al. 1997
Urine	Samples treated with HCl/methanol to convert HEAA to its methyl ester; samples then directly injected into GC-MS	GC-MS	0.07 µg/mL (1,4-dioxane); and 0.1 µg/mL (HEAA)	No data	Young et al. 1976

GC = gas chromatography; HCl = hydrochloric acid; HEAA = β-hydroxyethoxyacetic acid; MS = mass spectrometry

EPA Method 8015B is used to determine the concentration of 1,4-dioxane in environmental samples by GC. Samples may be introduced into the GC by direct injection (e g., aqueous samples) including the concentration of analytes by azeotropic distillation (EPA Method 5031). Purge-and-trap and solvent extraction are not appropriate for this method. Detection of the analyte is achieved by using a FID. Method detection limits for 1,4-dioxane in water, groundwater, and leachate are 12, 15, and 16 µg/L, respectively. Method detection limits for 1,4-dioxane in solids (e.g., incinerator ash and kaolin) are 0.31 and 0.16 mg/kg, respectively. Using azeotropic microdistillation, recoveries for 1,4-dioxane in groundwater, leachate, incinerator ash, and kaolin were 96–124, 102–103, 48–106, and 48–105%, respectively (EPA 1996a).

EPA Method 8260B is used to determine 1,4-dioxane in a variety of solid waste matrices by GC. This method is applicable to nearly all types of samples, regardless of water content, including various air sampling trapping media, groundwater and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments. Samples may be introduced to the capillary GC column by direct injection following dilution, sample concentration by azeotropic distillation (EPA Method 5031), and closed system vacuum distillation (EPA Method 5032) for aqueous, soil, oil, and tissue samples. Detection of the analyte eluted from the capillary column is achieved by using MS. The estimated quantitation limit for 1,4-dioxane is somewhat instrument dependent and also dependant on the choice of sample preparation/introduction method. No information on the recoveries for 1,4-dioxane were provided for this method (EPA 1996b).

EPA Method 1624 is used to determine 1,4-dioxane in water and in municipal and industrial discharges by isotopic dilution GC-MS. In this method, isotopically labeled 1,4-dioxane-d_8 is added to the sample as an isotope dilution standard. The samples are then introduced into the GC using a purge-and-trap methodology. 1,4-Dioxane is separated by GC and detected by MS. The labeled compounds serve to correct for the variability of the analytical technique. The detection limit for this method is 10 µg/L (EPA 2001).

Draper et al. (2000) described a sensitive method for detection of 1,4-dioxane in drinking water. This method was based on continuous LLE of 1,4-dioxane from aqueous samples by dichloromethane. Extraction of 1,4-dioxane in dichloromethane was followed by analysis using GC-MS. Detection limits as low as 0.2 µg/L were achieved in this method.

Epstein et al. (1987) described two methods for the determination of 1,4-dioxane in water and in solids and sediments. In the first method, 1,4-dioxane is extracted from water and soil samples using a heated purge-and-trap system following salting out with sodium sulfate at 1.6 M. GC-MS is then used as the method of analysis. The detection limit reported for this method was 2 ppb with recoveries averaging 85%. In the second method, 1,4-dioxane is adsorbed on coconut shell charcoal followed by desorption with carbon disulfide/methanol. Analysis of the desorbate is conducted by GC with flame ionization detection. The limit of quantitation is around 2 ppb with recoveries ranging from 63 to 129%.

Kadokami et al. (1990) described a method for analysis of 1,4-dioxane in water by GC-MS. Preconcentration of 1,4-dioxane is achieved by passing the aqueous sample through an activated carbon column followed by elution with acetone-dichloromethane. The organic extract is then concentrated with a Kuderna-Danish concentrator followed by direct injection into the GC-MS with a selective ion monitor. The method detection limit was reported to be 0.024 µg/L. Recoveries of 1,4-dioxane from organic-free water, seawater, and river water were 98–101, 102, and 101%, respectively. Kawata et al. (2001) described a similar method of analysis for 1,4-dioxane in water. However, in this method, the solid-phase extraction media was activated carbon fiber felt with acetone as the elution media. Analytical determination of 1,4-dioxane was accomplished by GC-MS detection. The method detection limit was reported to be 0.03 µg/L. Recoveries of 1,4-dioxane in groundwater and river water were 97 and 92%, respectively.

Analytical methods for the determination of 1,4-dioxane in environmental samples are given in Table 7-2.

7.3 OTHER SAMPLES

Several methods are available that may be used to determine 1,4-dioxane in food, consumer cosmetic products, and surfactant raw materials.

The concentration of 1,4-dioxane in food additives may be determined using the 1,4-dioxane limit test (Committee on Food Chemicals Codex 1996). 1,4-Dioxane is extracted from a sample placed in a closed-system vacuum distillation apparatus. The distillate is then analyzed using GC-FID. The detection limit was not specified for this method. Daniels et al. (1981) utilized a similar methodology in the analysis of

Table 7-2. Analytical Methods for Determining 1,4-Dioxane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Samples collected by drawing air through a solid sorbent tube containing coconut shell charcoal; 1,4-dioxane eluted with agitation using carbon disulfide	GC-FID	0.01 mg per sample	No data	NIOSH 1994 (NIOSH Method 1602)
Drinking water	Continuous liquid-liquid extraction using dichloromethane	GC-MS	0.2 µg/L	No data	Draper et al. 2000
Water, seawater, and river water	Pre-concentration by passing aqueous sample through an activated carbon column followed by elution with acetone-dichloromethane; organic extract concentrated with a Kuderna-Danish concentrator	GC-MS with a selective ion monitor	0.024 µg/L	98–102%	Kadokami et al. 1990
Groundwater and river water	Solid-phase extraction using activated carbon fiber felt with acetone as eluent	GC-MS	0.03 µg/L	97%, 92%	Kawata et al. 2001
Water, groundwater, leachate	Direct injection or azeotropic distillation (i.e., EPA Method 5031)	GC-FID	12–16 µg/L	96–124% (ground-water), 102–103% (leachate)	EPA 1996a (EPA Method 8015B)
Water, and municipal and industrial discharges	Isotopically labeled 1,4-dioxane-d_8 is added to the sample as an isotope dilution standard	Purge-and-trap GC-MS	10 µg/L	No data	EPA 2001 (EPA Method 1624)
Water, and solids and sediments	Adsorbed on coconut shell charcoal followed by desorption with carbon disulfide/methanol	GC-FID	2 ppb (µg/L or µg/kg)	63–129%	Epstein et al. 1987
Water, and solids and sediments	Extracted from water and soil samples using a heated purge-and-trap system following salting out with sodium sulfate at 1.6 M	GC-MS	2 ppb (µg/L or µg/kg)	85%	Epstein et al. 1987

Table 7-2. Analytical Methods for Determining 1,4-Dioxane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments	Azeotropic distillation (i.e., EPA Method 5031) or closed system vacuum distillation (i.e., EPA Method 5032)	GC-MS	No data	No data	EPA 1996b (EPA Method 8260B)

1,4-Dioxane-d_8 = deuterium labeled 1,4-dioxane (or $C_4D_8O_2$); EPA = Environmental Protection Agency; FID = flame ionization detector; GC = gas chromatography; kg = 10^3 grams; L = liter; mg = 10^{-3} grams; µg = 10^{-6} grams; MS = mass spectrometry; NIOSH = National Institute for Occupational Safety and Health; ppb = parts per billion

1,4-dioxane in the food additive, polysorbate 65. The detection limit was not specified. Recoveries for 1,4-dioxane concentrations ranging from 0.5 to 600 ppm were from 85 to 101%, respectively.

Stafford et al. (1980) described the determination of 1,4-dioxane in ethoxylated surfactants using a direct injection GC method. 1,4-Dioxane is extracted from the ethoxylated surfactants using chlorobenzene, which is then diluted, and injected directly into the GC-FID. The detection limit was reported to be 0.5 mg/kg with a recovery of approximately 100%. Rastogi (1990) reported method for the identification and quantification of 1,4-dioxane in polyethoxylated surfactants using headspace GC-MS. Dichloromethane and 1,4-dioxane-d_8 are added to the surfactant sample in a closed vial, which is then heated at 80 °C for 16–18 hours. The headspace gases are sampled with a gas-tight syringe and injected into the GC-MS for quantitative analysis. The detection limit for this method was approximately 0.3 ppm with recoveries of 92–94%.

1,4-Dioxane may be quantified in commercial cosmetic products by reversed-phase high-performance liquid chromatography (Scalia et al. 1990). Cosmetic samples are extracted using solid-phase extraction cartridges. Samples are then analyzed directly on a reverse-phase column with spectrophotometric detection at 200 nm and acetonitrile-water as eluent. The limit of detection was reported to be 6.5 µg/g. The recovery of 1,4-dioxane was between 81.5 and 90.1% in the 30–90 µg/g range. Ghassempour et al. (1998) described a modified GC-MS method for determination of 1,4-dioxane in cosmetic products (i.e., polyoxyethylene compound). Cosmetic product samples are prepared by dissolution of the material in dichloromethane. Samples are then analyzed directly by injection into a programmable temperature vaporizer attached to GC-MS. The minimum detection limit was reported to be 1 ng/L for this method. However, as this value is very low, the detection limit is likely much higher than reported by the authors.

Analytical methods for the determination of 1,4-dioxane in food and food additives, cosmetics, and ethoxylated surfactant samples are given in Table 7-3.

7.4 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 1,4-dioxane is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research

designed to determine the health effects (and techniques for developing methods to determine such health effects) of 1,4-dioxane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.4.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Existing methods do not appear to be sensitive enough to measure background levels of 1,4-dioxane and its metabolite, HEAA, in the population (Young et al. 1976). Standard methods for the determination of 1,4-dioxane and its metabolite, HEAA are needed to determine whether the general population is exposed to 1,4-dioxane.

Effect. Existing methods appear to be sensitive enough to measure levels of 1,4-dioxane and its metabolite, HEAA, at levels at which biological effects may occur in humans (Young et al. 1976). However, more precise, accurate, and reliable methods would be useful to determine levels of biological effects.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The purpose of analytical methods is to identify contaminated areas and to determine if contaminant levels constitute a concern for human health. The media that are of most concern for human exposure to 1,4-dioxane are drinking water, food, and cosmetic products. In water, there are methods sensitive enough to measure background levels in the environment down to the sub-ppb level (<1 µg/L) (Draper et al. 2000; Kadokami et al. 1990; Kawata et al. 2001). Standard methods are also available for measurement of 1,4-dioxane in air and water samples (EPA 1996a, 1996b; NIOSH 1994). Methods have also been reported for the determination of 1,4-dioxane in food and food additives (Committee on Food Chemicals Codex 1996; Daniels et al. 1981), cosmetics (Ghassempour et al. 1998; Scalia et al. 1990), and ethoxylated surfactant materials (Rastogi 1990; Stafford et al. 1980). Additional or improved methods

that offer increased sensitivity would be useful for determining sub-ppb (<1 µg/L) levels of 1,4-dioxane in foods and food additives, which would be helpful in determining whether exposure to 1,4-dioxane in food is significant for the general population.

7.4.2 Ongoing Studies

The Environmental Health Laboratory Science Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of 1,4-dioxane and other volatile organic compounds in blood. These methods use purge-and-trap methodology, high resolution gas chromatography, and magnetic sector mass spectrometry, which give detection limits in the low parts per trillion (ppt) range.

No ongoing studies on 1,4-dioxane were found as a result of a search of the Federal Research in Progress (FEDRIP 2004).